Ultraviolet-induced histological and histochemical changes in the integument of newly molted American cockroaches, *Periplaneta americana* (Dictyoptera: Blattaria: Blattidae)

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Hypodermal cells of newly molted cockroaches irradiated with UV (254 nm) are progressively destroyed, with first evidence of structural damage occurring 2 h after initiation of irradiation. Before that, melanin formation begins to fall and irradiated animals become progressively more deficient in melanins or their precursors. Proteins of the exocuticle fail to tan in irradiated cuticle, as indicated by continued uptake of protein stain at a time when unexposed cuticle becomes refractive to staining. Hypodermal cells are irreversibly damaged by UV irradiation, as evidenced by lack of endocuticle production. Similarly, tonofibrillae are progressively destroyed in tergal attachments of tergosternal muscles exposed to UV. Muscles become detached, possibly in the legs as well, resulting in sluggish behavior. With tanning incomplete and hypodermal cells destroyed, the integument ceases to become an effective barrier to dehydration, and molting failure or death, due to desiccation, may result.

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L'irradiation à l'UV(254 nm) chez les blattes, tout de suite après la mue, détruit progressivement les cellules hypodermiques; les premiers symptômes de dommages structuraux apparaissent 2 h après le début de l'irradiation. Avant cela, la formation de la mélanine subit un ralentissement et les animaux irradiés souffrent d'une déficience progressive des mélanines ou de leur précurseur. Les protéines de l'exocuticule irradiée ne subissent pas de tannage, car on assiste à une continuation de l'absorption du colorant des protéines à un moment où une cuticule normale devient réfractaire au colorant. Le dommage causé par l'UV aux cellules hypodermiques est irréversible, ce qui se manifeste par l'absence de production de l'endocuticule. De même, à l'UV, les tonifibrilles des fibres rattachées aux tergites dans les muscles tergo-sternaux s'atrophient. Les muscles des pattes se détachent probablement aussi, car on observe un comportement léthargique. A cause du tannage incomplet et de l'atrophie des cellules hypodermiques, le tégument cesse d'être une protection efficace contre la déshydratation; on peut alors assister à l'impossibilité de muer ou à la mort par dessiccation. [Traduit par le journal]

Introduction

Karlson and Ammon (1963), Beard (1973), Cohen *et al.* (1973), and Gingrich (1974), have demonstrated that exposure of untanned insect cuticle to ultraviolet radiation (UV) curtails melanization and tanning. However, no explanations have been made on the mode of action of UV in producing these effects.

It is known that UV induces mutation in chromosomes of insects (Altenburg 1934; MacKenzie and Muller 1940; and Murikami 1970). Similarly, UV irradiation can induce a wide range of somatic effects from cytoplasmic and nuclear coagulation in Protozoa (Kimball 1955) to nuclear membrane thickening in human and rat cells (Erskine 1959).

The objective of this study was to determine histological and histochemical changes induced by UV irradiation of the American cockroach, *Periplaneta americana* (L.).

Materials and Methods

UV Irradiation of Insects

Newly molted large instars (8–10th) and adults were selected within 15 min after the molt (white stage). For histological and histochemical studies the left dorsum of the cockroach was covered with black electrical tape while the right dorsum was left uncovered. Irradiation was carried out for selected periods from 0.5 to 5.0 h with cockroaches held in greased beakers at a distance of 23 cm from the UV source. The source of UV radiation was twin 15-W germicidal lamps[1] emitting 2820 ergs s^{-1} cm^{-2} of 254 nm UV at a distance of 23 cm.

Histological and Histochemical Methods

After exposure to UV for selected periods up to 5 h and removal of heads and legs, animals were immersed in alcoholic Bouin's fixative overnight. Alcoholic dehydration, clearing, and embedding were performed according to standard histological procedures followed by microtomy of the entire abdomen in sections of 8 to 10 microns (μ). For histological studies, Mallory's triple stain or Heidenhain's iron hemotoxylin was used. In his-

[1]Sylvania Lighting Center, Danvers, Massachusetts.

tochemical tests, slides were treated separately in groups of 5–10 with ammoniacal silver nitrate (for melanin), 2% aqueous ferric chloride (either for *o*-diphenols or as a bleaching agent for melanin), or with Millon's reagent for tyrosine according to standard methods of Pearse (1953). Ratings were made on staining intensity of exo-, meso-, and endo-cuticle from 1+ (weak) to 4+ (very strong).

Results and Discussion

General Appearance and Behavior of Irradiated Cockroaches

Newly molted individuals, selectively irradiated on one side, darkened only on the protected side (Fig. 1). Tanning, as well as darkening, was inhibited by UV irradiation as shown by the reduction in cuticular tensile strength by more than 50% compared with normally tanned nymphs (Gingrich 1974). Irradiation of fully tanned nymphs often resulted in sluggish behavior that required repeated prodding to stimulate movement. Dorsally, the tergites of such nymphs often appeared blackened and lacked the waxy sheen of unexposed nymphs. Additionally, fully tanned, UV-irradiated nymphs frequently died in attempting to escape the exuvium at the next molt (Fig. 2).

Histological and Histochemical Alterations

One-half hour after the initial exposure there were no apparent differences in staining reactions of the cuticle (Table 1). Judging by the spotty acid fuchsin and hematoxylin staining in the exo- and meso-cuticles (inner exocuticle), protein was still being incorporated into these areas. Silver nitrate reduction was slight (1 to 2+) in exo- and meso-cuticles of protected and irradiated sides, signifying that melanin formation was just beginning. The first differences in silver nitrate reduction occurred 1 h after the molt (Table 1), the exocuticle of the irradiated side being slightly less reactive. That silver nitrate reduction was largely monitoring melanin or melanin precursors was indicated by the following: (1) treating reduced silver nitrate sections with ferric chloride resulted in nearly complete bleaching, as indicated for melanins by Pearse (1953), (2) ferric chloride treatment in the absence of silver nitrate reduction resulted in a negative test for *o*-dihydroxyphenols (Dennell and Malek 1955), and (3) Millon's reaction for tyrosine was very weak even when silver nitrate reduction was very strong.

At 1.5 h after the molt there were still no differences in acidophilic staining (Table 1) on the two sides of the animal, although the exocuticle gave stronger reactions than the mesocuticle, suggesting that protein was still being incorporated into these areas. Silver nitrate reduction was very strong (4+) in the protected exocuticle, less strong in irradiated exocuticle (3–4+), while the mesocuticle showed moderate reduction (2+) on both sides (Table 1).

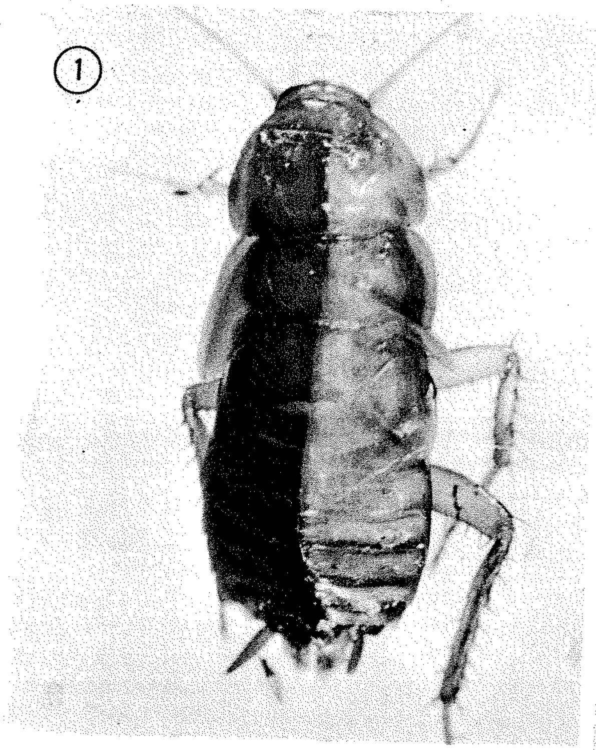
The first changes in hypodermal cells were seen 2 h after the molt, with irradiated cells showing granulation, increased numbers of chromatic globules (staining with orange G), disrupted tonofibrillae, increased vacuolization of cytoplasm, and rupturing of some cells (Fig. 4). Similarly, irradiated mesocuticle displayed irregular patches of acidophilic material while in the protected mesocuticle it was much more diffuse. The exocuticle of both sides was strongly acidophilic, suggesting that tanning had not begun appreciably (Table 1). Silver nitrate reduction remained the same as at 1.5 h (Table 1).

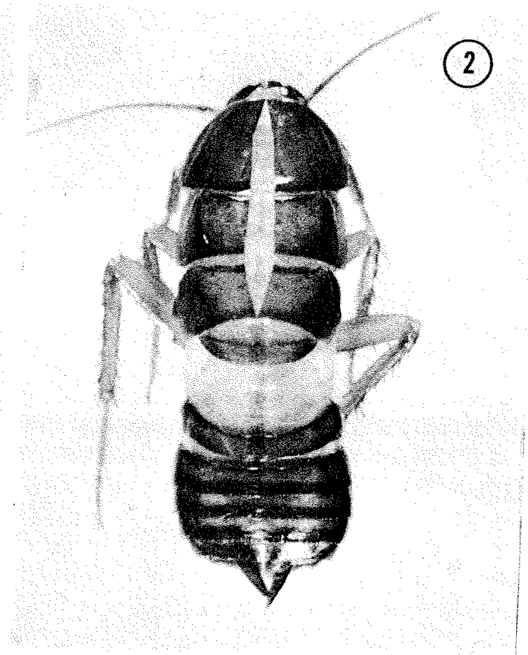
The next major change occurred 4 h after the molt, when the protected exocuticle failed to stain with acid fuchsin but continued to stain with hematoxylin, in contrast to the findings of Dennell and Malek (1955), who observed that hematoxylin affinity was lost first from the exocuticle. The loss of acidophilia in the exocuticle was interpreted as a result of protein tanning, the timing of which agrees with that projected by Dennell and Malek (1955).

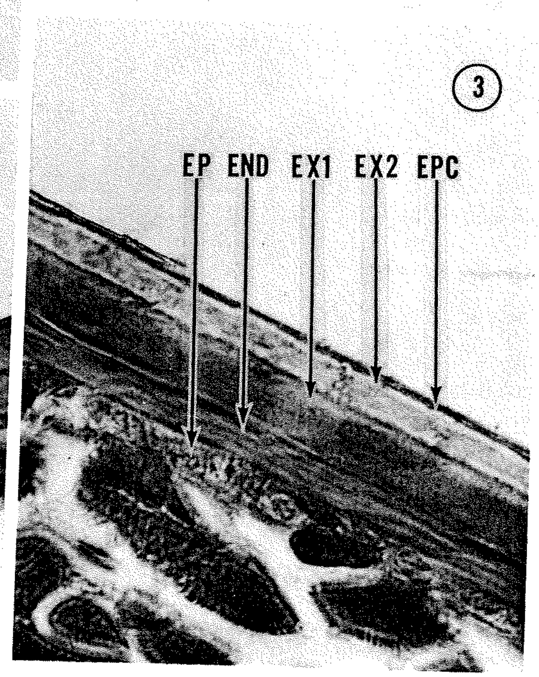
Five hours after the molt, the silver nitrate reaction was actually stronger in irradiated mesocuticle than in the protected mesocuticle, suggesting that more reducing protein was present in the irradiated mesocuticle (Table 1). Acidophilia continued to be absent only from the protected exocuticle.

One, 3, and 18 days after the molt, acidophilia was still strong in the irradiated exocuticle, indicating that tanning had never occurred. Additionally, the endocuticle was never elaborated on the irradiated side, suggesting that the hypodermal cells were irreversibly damaged (compare Figs. 3 and 4).

UV irradiation also resulted in damage to muscle attachments. A large nymph, irradiated for 3 h on the right half, displayed damage to the tergal attachments in four of the seven observed tergosternal muscles of the irradiated abdomen, with no such damage seen in the protected abdomen. Two of the damaged muscles had broken and fragmented tonofibrillae while the other two were completely pulled away from the cuticle along with parts of the cuticle and hypo-







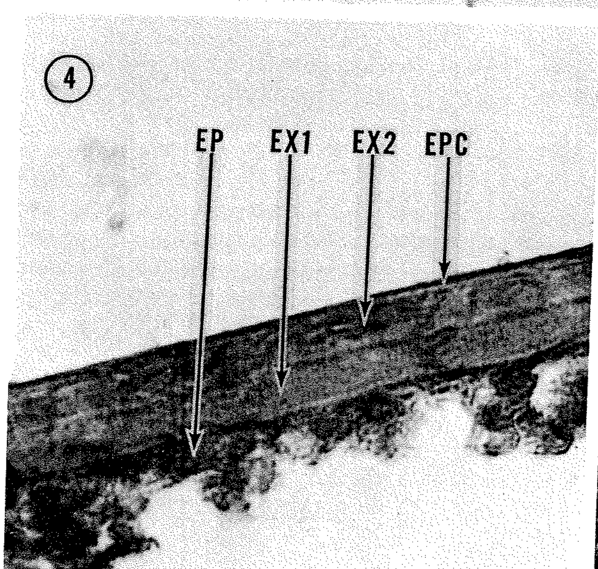


FIG. 1. Cockroach irradiated with UV (4 h) on one side (light half) while being protected on the other side (dark half) during the tanning process. Photograph taken 24 h after the molt. FIG. 2. Cockroach attempting to molt after UV irradiation but unable to escape the exuvium. FIG. 3. Cockroach cuticle protected from UV irradiation during the tanning process. Note the five distinctly separated layers, viz. epicuticle (EPC), epidermis (EP), endocuticle (END), inner exocuticle (mesocuticle = EX 1), and outer exocuticle (EX 2). Staining: Mallory's triple stain. Preparation made 3 days after the molt. × 1174. FIG. 4. Cockroach cuticle exposed to 4 h UV during the tanning process. Note the granular and irregular appearance of the epidermis (EP), and indistinct separation of inner exocuticle (mesocuticle) and outer exocuticle (EX 1 and EX 2). The endocuticle (END) is lacking, epicuticle (EPC) appears normal. Staining: Mallory's triple stain. Preparation made 3 days after the molt. × 1174.

TABLE 1

Histological and histochemical staining reactions of exocuticle (1), mesocuticle (2), and hypodermis of newly molted cockroaches irradiated on one side (Exp) with 254 nm UV (2820 ergs s^{-1} cm^{-2}) and protected on the other side (Pro) for selected intervals of 0.5 to 5 h. Some animals were not sacrificed until 1, 3, or 18 days after exposure. Staining was via acid fuchsin, iron hematoxylin, or ammoniacal silver nitrate. Reactions of anterior abdominal segments (ant = second to fourth tergites) or posterior abdominal segments (post = sixth to ninth tergites) ranged from negative (−) to weak (+) to very strong (4+)

Time after molt	Location in animal	Stain: Fuchsin 1	Fuchsin 2	Hematoxylin 1	Hematoxylin 2	Silver nitrate 1	Silver nitrate 2	Hypodermis
0.5 h	Pro ant	2–3+	2+	Black (3+)		2+	2+	Normal
	Pro post	2–3+	2+	patches and		2+	1–2+	
	Exp ant	2–3+	2+	unstained		2+	2+	
	Exp post	2–3+	2+	areas (−)		2+	1–2+	
				in both layers				
1 h	Pro ant					3–4+	2+	Normal
	Pro post					3–4+	+	
	Exp ant					3–4+	2+	
	Exp post					2–3+	+	
1.5 h	Pro ant	3–4+	2+	4+	Black (3+)	4+	2+	Normal
	Pro post	3–4+	2+	4+	and unstained	4+	2+	
	Exp ant	3–4+	2+	4+	(−) areas	3–4+	1–2+	
	Exp post	3–4+	2+	4+	interspersed	2–3+	1–2+	
2 h	Pro ant	4+	3+	4+	2–3+	4+	2+	Granules in
	Pro post	4+	3+	4+	Patches	4+	1–2+	cells, chromatic
	Exp ant	4+	2–3+	4+	of black	3–4+	2+	globules, vacuolized
			patches		and unstained			cells, detaching
	Exp post	4+	3+	4+	areas	2–3+	1–2+	tonofibrillae
					(−) interspersed			
3 h	Pro ant	4+	3+			4+	1–2+	More chromatic
	Pro post	4+	3+			4+	1–2+	globules, granules
	Exp ant	4+	2–3+			3+	2+	in cells, vacuolized
			patches					cytoplasm,
	Exp post	4+	3+			3–4+	2+	detached tonofi-
								brillae
4 h	Pro ant	−	3+	4+	−	3–4+	2+	More disrupted
	Pro post	−	3+	4+	−	4+	2+	cells; coagulated
	Exp ant	3+	3+	4+	−	2+	2+	cytoplasm
	Exp post	3+	3+	4+	−	2–3+	2+	
5 h	Pro ant	−	3+			4+	2+	
	Pro post	−	3+			4+	2+	
	Exp ant	3+	3+			4+	3+	
	Exp post	3+	3+			4+	3+	
24 h	Pro ant	−	3+					Cells compacted,
	Pro post	−	3+					cytoplasm coagulated
	Exp ant	3+	3+					or disrupted into
	Exp post	3+	3+					granules
3 days	Pro ant	−	3+	−	3+			Cells compacted,
	Pro post	−	3+	−	3+			appeared
	Exp ant	3+	3+	3+	3+			degenerative
	Exp post	3+	3+	3+	3+			
18 days	Pro ant	−	3+	−	2+			Cells compacted,
	Pro post	−	3+	−	2–3+			appeared
	Exp ant	3+	3+	3+	3+			degenerative
	Exp post	3+	3+	3+	3+			

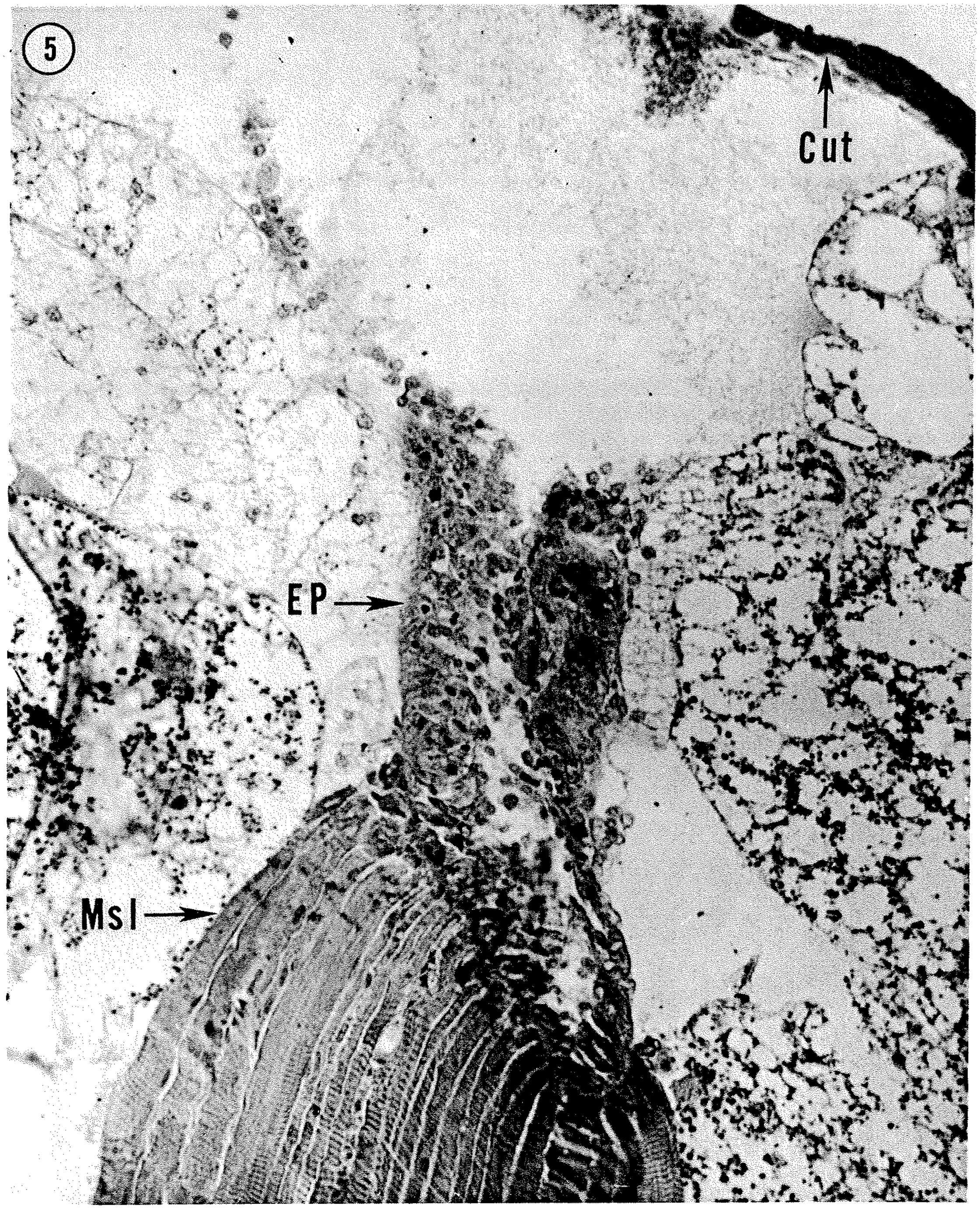


FIG. 5. Cockroach irradiated as in Fig. 1. Note the muscle (Msl) pulled away from the cuticle along with epidermal cells (EP) and cuticular fragments (Cut). Staining: Mallory's triple stain. × 600.

dermis (Fig. 5). Of seven specimens examined, five showed tergal muscle detachments or tonofibrillar damage on the irradiated side only, thereby precluding the possibility of chemical fixation artifacts. The other two, exposed for less than 2 h, showed no damage to muscle attachments of either side.

Consequences of UV-induced Damage

The destruction of tergosternal muscle attachments suggests that other muscle attachments might also be affected. The general immobility and awkwardness of UV-exposed cockroaches indicates that leg muscle attachments might be broken. In this case, affected individuals would be severely handicapped in the competition for food, water, and mates. Irreversible damage to hypodermal cells, as evinced by absence of endocuticle and waxy secretions in irradiated animals, and tanning failure in the exocuticle signify that the integumental barrier to the environment has been badly compromised. Expected consequences would be rapid dehydration and increased demand for food and water at a time when mobility may be limited. Ultimately, dehydration and starvation could become critical, as has often been observed. Destruction of hypodermal cells might also result in critical shortages of molting fluid and thus account for molting failures as seen in Fig. 2.

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